



PATENT

ATTORNEY DOCKET NO. 81289-294309

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant : Timothy A. Hovanec                      Art Unit : 1651  
Serial No. : 10/659,948                              Examiner : Marx, Irene  
Filed : 09/10/2003  
Title : METHOD OF USING AMMONIA-OXIDIZING BACTERIA

Assistant Commissioner for Patents

Washington, DC 20231

**DECLARATION UNDER 37 C.F.R. §1.132**

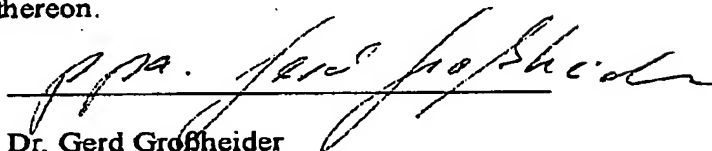
Dear Sir/Madame:

I, Dr. rer. nat. Gerd Großheider, do hereby declare and state that:

1. I am the director of research and development of Tetra GmbH, a company of Spectrum Brands, parent company of the Applicant and producer of the nitrifying bacteria the invention is referring to.
2. I am familiar with the subject matter described and claimed in United States Patent Application Serial No. 10/659,948, filed September 10, 2003, entitled: "METHOD OF USING AMMONIA-OXIDIZING BACTERIA."
3. I am familiar with the prosecution history of Application Serial No. 10/659,948.
4. The inventor of the subject matter described and claimed in Application Serial No. 10/659,948 is no longer an employee of the Applicant.
5. I understand that the Examiner is rejecting the present claims under 35 U.S.C. 112 as missing the critical step of culturing or growing the bacterial cells in order to oxidize ammonia to nitrite in the process as claimed.

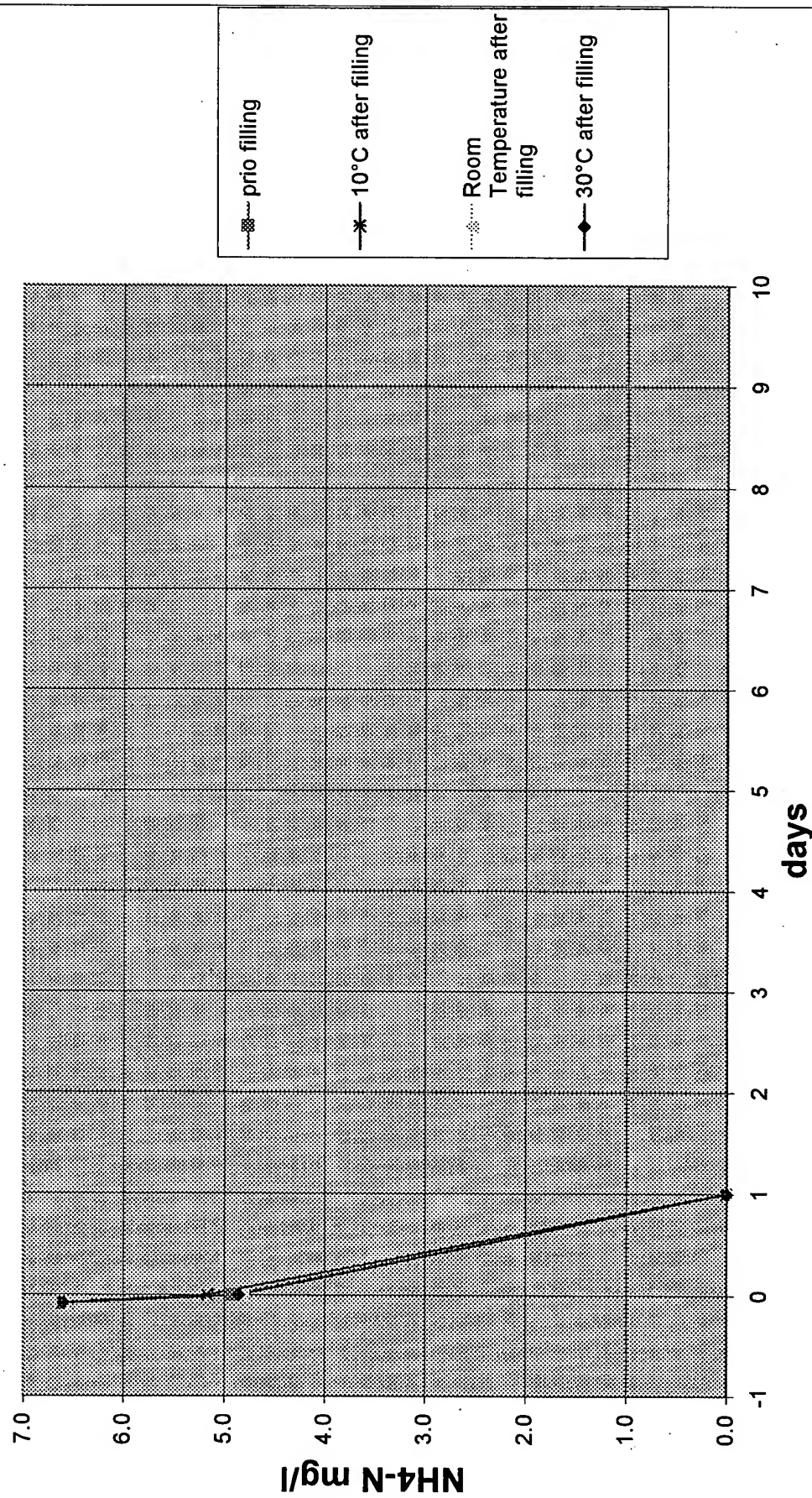
6. I respectfully submit that there is no bacterial growth of the claimed bacterial strain necessary after introduction of the claimed amount into the aquarium for the nitrification process to occur.
7. I respectfully submit that the included experimental data in Exhibit 1, including the chart "Ammoniachloride/Nitrite kinetics in a breeding tank," shows that nitrification is immediate upon introduction into the aquarium.
8. I respectfully submit that the performance of the bacteria as claimed, identified as BioSpira, was evaluated by a "Flask-Test" method (Exhibit 2) performed in laboratory and is a test commonly used in the field to evaluate how the concentration of the substrate  $\text{NH}_2\text{Cl}$  is transformed into nitrite and later from nitrite into nitrate.
9. I respectfully submit that nitrifiers, such as the claimed bacteria, need about 12-14 hours per propagation cycle, and that this rate does not correlate with the nitrification rate as our test results demonstrate that nitrification is immediate upon introduction into the aquarium of the claimed bacterial strain and that the entire nitrification process is completed in less than 1 day.
10. I respectfully submit that the claims expressly require introducing an amount sufficient to operate as claimed and that once that amount of bacterial strain is introduced there is no further growing or culturing necessary within the aquarium for nitrification to occur.
11. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 30.09.2007

  
Dr. Gerd Großheider

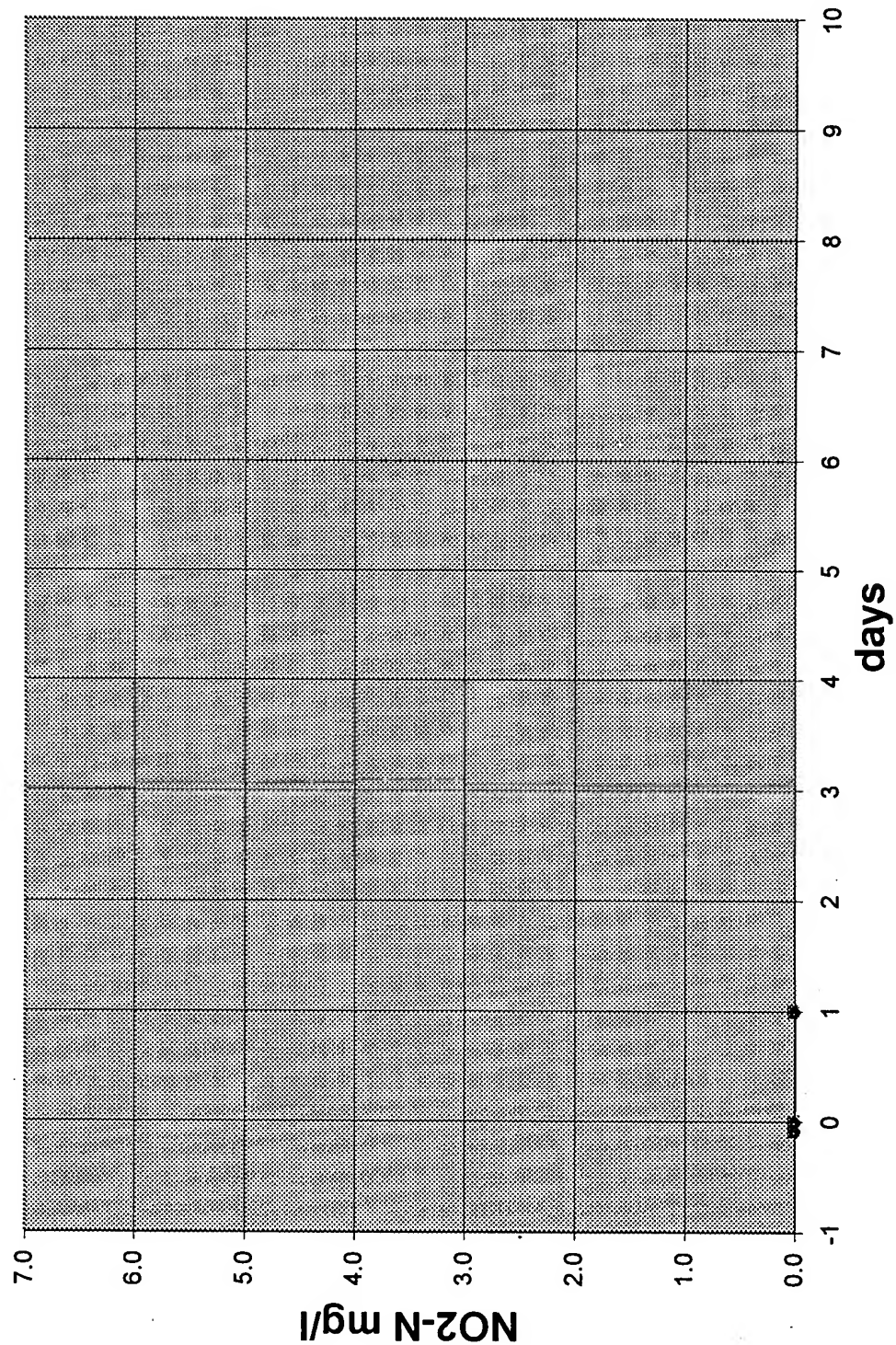
## Erlenmeyertest 10, Charge 148563

### Ammoniumabbau



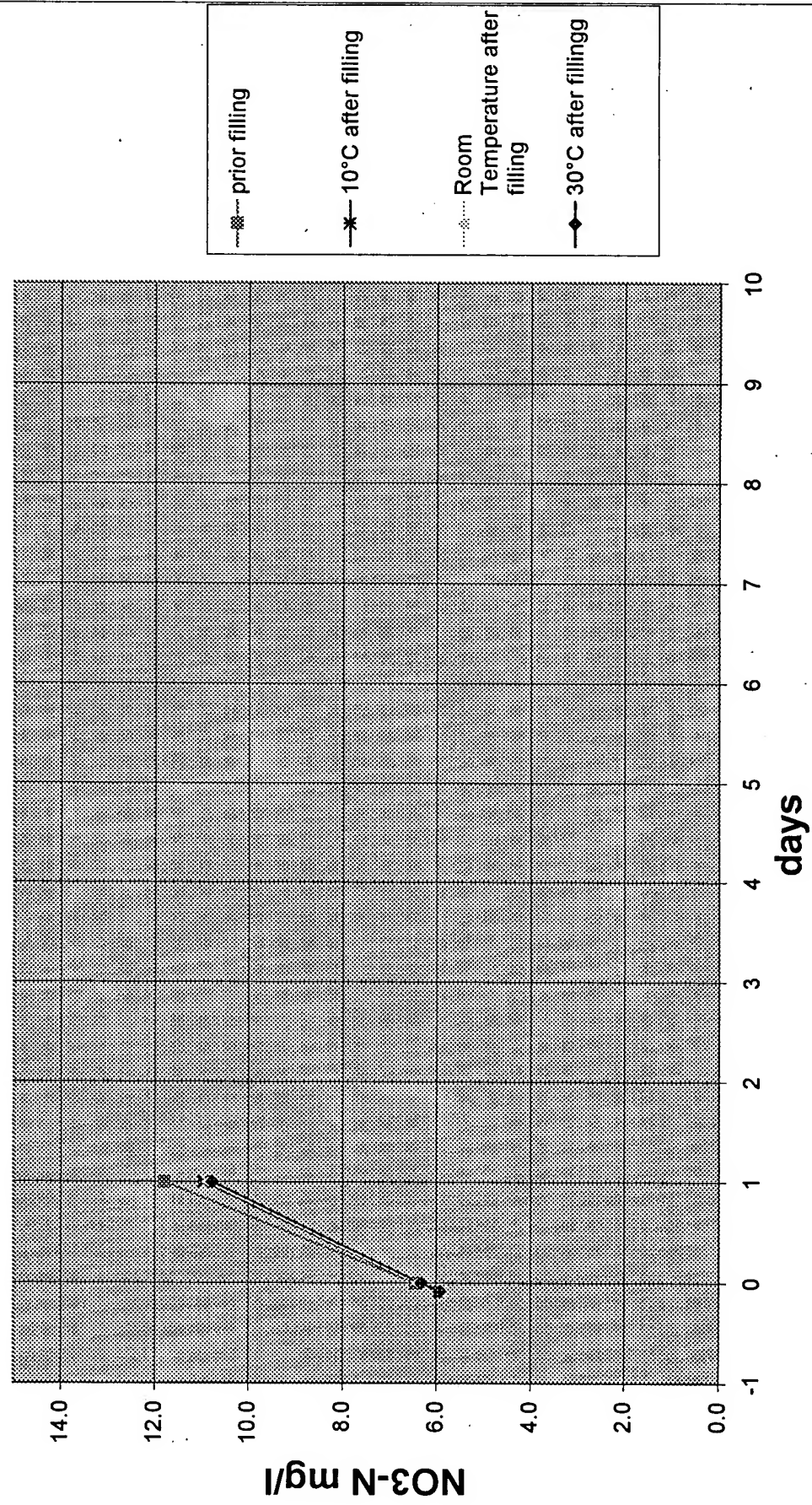
## Erlenmeyertest 10, Charge 148563

### Nitrite Formation over Time



## Erlenmeyertest 10, Charge 148563

### Nitrate Formation

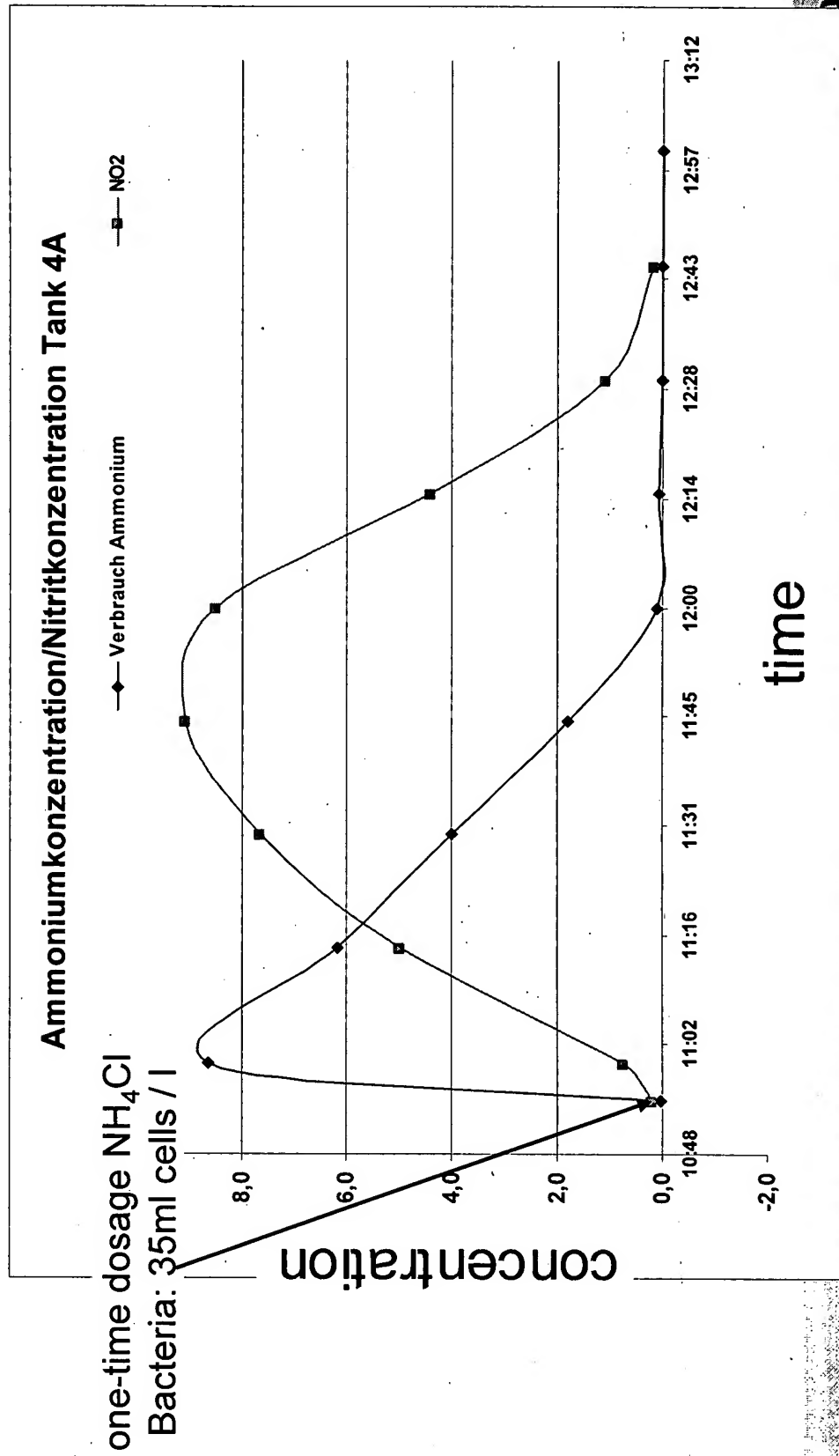




UNITED PET GROUP, INC



## Ammoniumchloride / Nitrite kinetics in a breeding tank



## Ammonia and Nitrite Oxidation Flask Test for BioSpira QC for CRD

### Materials needed:

- 50 mg/ml ammonia-N Ammonium Chloride Stock: (19.24g ammonium chloride in one liter deionized water)
- (Sodium bicarbonate)
- 500 ml Erlenmeyer flasks:
  - 2 replicates of each treatment
  - Treatments are:
    - BioSpira (test bacteria), application for 120L aquarium tank tested at 140ml per 1L
    - Negative Control (no bacteria)
- Stir plate (preferably a multi-position stir plate so all replicates are treated the same)
- stir bars
- 10 ml Pipetman
- 0.20µm syringe filters
- 10ml syringe
- Sample tubes
- Graduated cylinders
- Containers to hold water
- BQS Tank Water
- Method and equipment to measure ammonia-N, nitrite-N, and nitrate-N
- pH Meter

### Method:

1. Thoroughly clean 500 ml Erlenmeyer flasks and sterilize it 2 hours at 200 °C.
2. Number flasks. Randomly assign each flask number a treatment.
3. Make a 3 L solution of 5 mg/L ammonia-N by diluting 3 ml 50 mg/ml ammonia-N stock solution to 3 L with BQS Tank Water (in a beaker).
4. Measure the pH value. (Add sodium bicarbonate until pH is ~8. Make sure solution is mixed well.)
5. Take two baseline samples from the beaker, measure with IC
6. Using a graduated cylinder, aliquot 200 ml of the 5 mg/L ammonia-N solution to each flask.
7. Add 1.0 ml of well mixed BioSpira to appropriate flask.
8. Add stir bars to each flask and place flasks on stir plate. Spin at medium-low speed for mixing and oxygenation. Cover the top of the flasks with a cap.
9. **Sample each flask in 24 hour intervals for 10 days.**
  - a. Using 10ml Pipetman, remove 10 ml from each flask and put it in a 30 ml plastic beaker.
  - b. For **Anion IC** take 4 ml with a syringe and filter it through a 5 µm filter, IC-H<sup>+</sup> cartridge and a 0.2 µm filter into a sample tube.  
Syringe, 5 µm filter, IC-H<sup>+</sup> cartridge and a 0.2 µm filter will be cleaned with deionized water after each sample.
  - c. For **Kation IC** add 2 drops of 1 M HNO<sub>3</sub> into the plastic pitcher, mix it carefully, take it all into a syringe and filter it through a 5 µm filter and a 0.2 µm filter into a sample tube.  
Syringe, 5 µm and a 0.2 µm filter will be cleaned with deionized water after each sample.

### Acceptance Criteria:

Ammonia-N should reach 0mg/L by day 5.

Nitrite-N should reach 0mg/L by day 8.